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QUALITATIVE CHANGES IN THE THIRD SERUM COMPONENT.*†

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IN work begun about three years ago for the purpose of testing the application of certain physico-chemical laws to hemolytic serum, a number of apparently paradoxical results were observed.[1] In attempting to determine the cause of these seeming paradoxes, the discovery was made that direct analytical methods are not applicable to many quantitative serum determinations.[2]

The application of quantitative methods is one of the most fundamental problems in any field of biological chemistry. It is the first problem that should be solved, before quantitative work is begun in that field. Failure to solve this problem may lead to experimental error and to erroneous conclusions. Work was therefore undertaken to determine why ordinary methods of analysis are not applicable to the phenomena in question, and, if possible, to find an indirect method applicable to them.

The problem immediately at hand was the measurement of the absorption of hemolytic amboceptor by blood corpuscles. Present analytical methods are not applicable to this problem, because the heated hemolytic serum, used for amboceptor measurements, is so changed by contact with corpuscles, that duplicate titrations do not agree.[3]

It was found that one of the reasons for this non-agreement of duplicate analyses was the probable change in the relative amounts of amboceptor and third component in such serum during exposure.[4] But the changes produced in a serum by altering the relative amounts

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[1] *Jour. Infect. Dis.*, 1905, 2, p. 490; *Centralbl. f. Bakt.*, 1906, 40, p. 382.

[2] *Jour. Infect. Dis.*, 1905, 2, p. 493; *Jour. Biolog. Chem.*, 1906, 1, p. 213; *Centralbl. f. Bakt.*, 1906, 40, p. 386; *Trans. Chic. Path. Soc.*, 1905, 6, p. 319.

[3] *Jour. Infect. Dis.*, 1908, 1, p. 67.

[4] *Jour. Infect. Dis.*, 1906, 3, p. 648.

of these two substances artificially, was in no case as pronounced as the changes observed in the same serum after exposure. It was therefore suspected that, in addition to such possible quantitative changes,

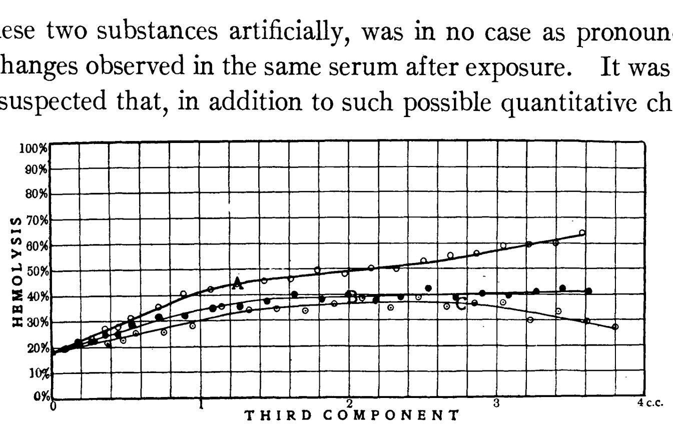

FIG. 1.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A curve showing the changes in hemolytic power produced by increasing amounts of third component (heated normal goat serum) when added to a constant amount of hemolytic goat serum. *B* and *C*= curves showing changes produced by the same third component after contact with sheep corpuscles. The number of corpuscles used for curve *C* was greater than that for curve *B*. The constant amount of hemolytic serum used in the experiment was capable, in itself, of producing 18 per cent hemolysis. Curves *B* and *C* show a decrease in auxilytic power, due to exposure to corpuscles.

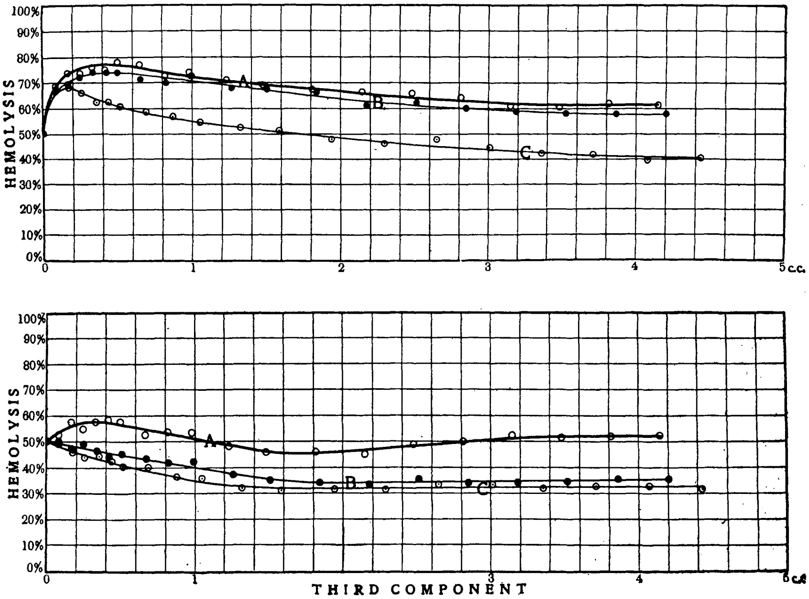

FIG. 2.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A =unexposed third component; *B* and *C*=exposed third component. Curves otherwise similar to those of Fig. 1. Curve C, of the upper set, shows the auxilytic power of unexposed third component replaced by an antilytic power. Curve *C* of the lower set shows a normal antilytic power increased after exposure.

there were changes of a qualitative nature as well. Can such changes be demonstrated?

In order to get a clew as to the nature of such possible changes, a study was made of the effect of exposing third serum component to sheep corpuscles. To do this, accurately measured quantities of third component[1] were allowed to stand in contact with carefully washed sheep corpuscles, under the same conditions and for the same length of time as in the original amboceptor experiments.[2] The third component was then freed from the corpuscles by centrifugation, and its effect on hemolysis compared with that of the original component,[3] kept under identical conditions, except for the contact with corpuscles. Data, so obtained, are shown graphically in Figs. 1, 2, 3, and 4.

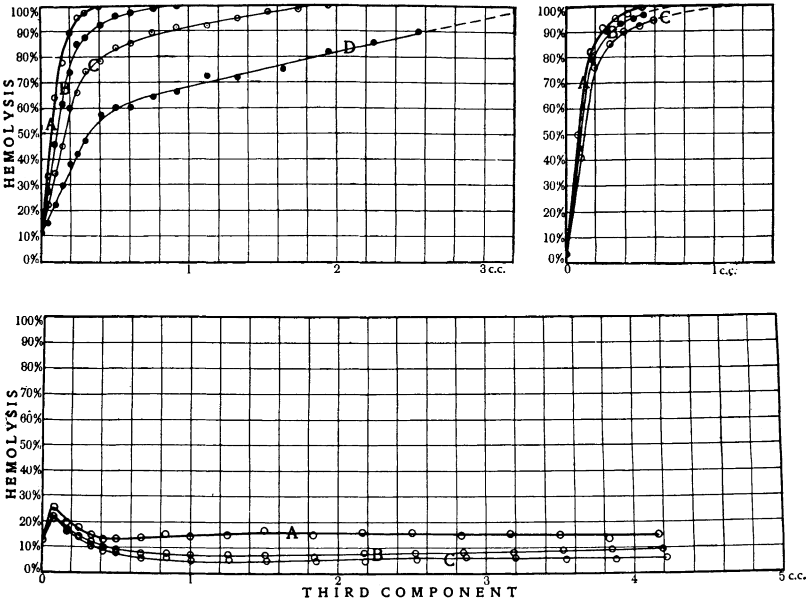

FIG. 3.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A = unexposed third component; *B*, *C*, and *D* = exposed third component. Curves otherwise similar to those of Figs. 1 and 2.

From these figures it is seen that exposure to corpuscles in all cases produces changes in the third component; that in every instance a third component that is originally auxilytic (hemolysis-increasing) has its auxilytic power decreased by such exposure, or even replaced by an antilytic power; and that a third component that is originally antilytic has its antilytic powers increased.

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[1] Heated normal serum, containing neither amboceptor nor complement.

[2] For material and technic, see *Jour. Infect. Dis.*, 1905, 2, p. 461.

[3] For the action of the third component, see *Jour. Infect. Dis.*, 1906, 3, p. 647.

Three hypotheses can be put forward to account for this change: First, that certain hemolytically active substances are absorbed from the third component, by the corpuscles, during such exposure. Second, that certain hemolytically active substances are given off into the third component, from the corpuscles, during the exposure. And, third, that the corpuscles produce, independent of such absorption or giving off of products, chemical changes in the third component, presumably by the action of the enzymes they contain. Attempt was made to test these three hypotheses.

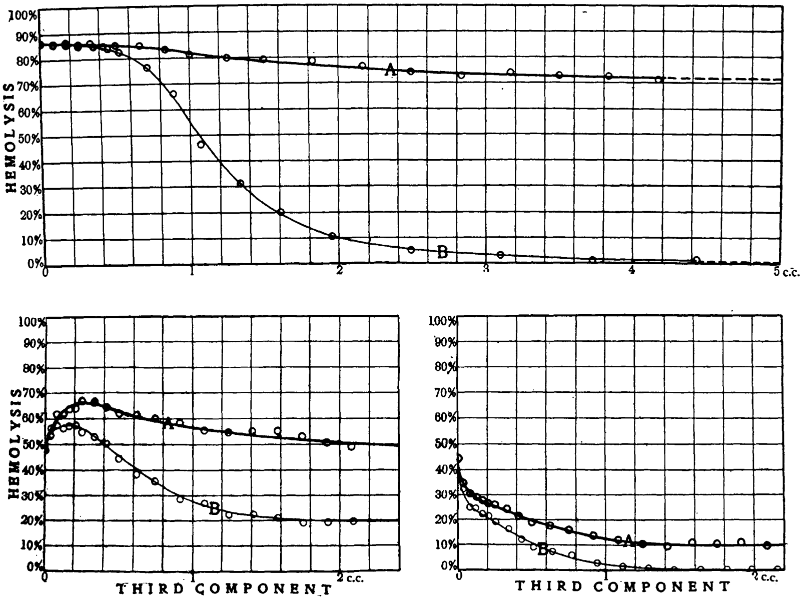

FIG. 4.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A=unexposed third component; *B*=exposed third component. Curves otherwise similar to those of Figs. 1, 2, and 3.

In order to determine whether or not active substances are absorbed by the corpuscles from the third component carefully washed corpuscles were exposed to third component, under conditions identical with those of the amboceptor absorption experiments above, were then freed from the third component by centrifugation, repeatedly washed in salt solution, and their susceptibility to hemolysis compared with that of unexposed corpuscles, prepared, kept, and washed under identical conditions, except for the contact with third component.

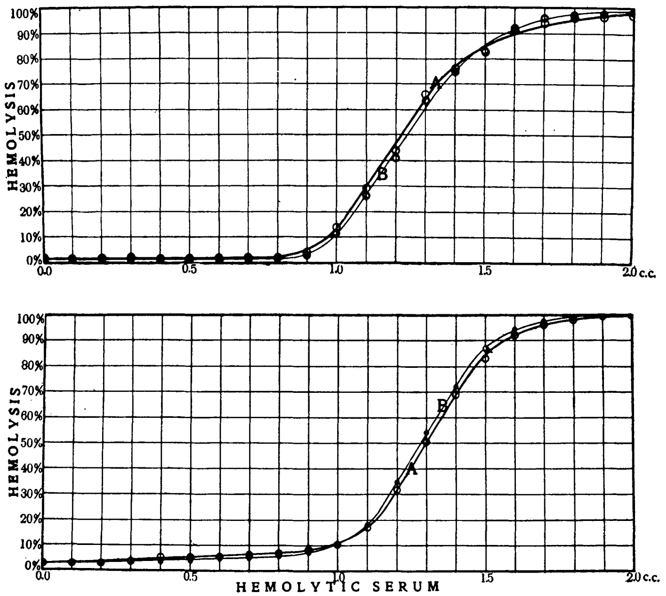

FIG. 5.—HEMOLYTIC SUSCEPTIBILITY OF CORPUSCLES AFTER EXPOSURE TO THIRD COMPONENT.

Each curve shows the percentages of hemoglobin liberated from a constant number of corpuscles, by increasing amounts of hemolytic serum. *A* = curves with unexposed corpuscles; *B* = curves with corpuscles after exposure to third component. The *B* curves show no change in hemolytic susceptibility after such exposure, within the limits of the experimental error. In the upper experiment the corpuscles were exposed to a powerfully auxilytic third component; in the lower experiment, to a third component practically inert.

The data from four such comparisons are given graphically in Figs. 5 and 6.

In the first two comparisons (Fig. 5), the corpuscles were found unchanged in hemolytic susceptibility, after exposure to third component, within the limits of the experimental error. In the second two comparisons (Fig. 6), changes were observed in hemolytic susceptibility, but in each case these slight changes were the opposite of those that would have followed a retention of third component by the corpuscles.[1]

[1] The slight changes observed in these corpuscles will be the subject of further investigation.

We are therefore obliged to conclude that the hemolytically active substances of the third component are either not absorbed by corpuscles, or, if absorbed, are held in such loose chemical combination that

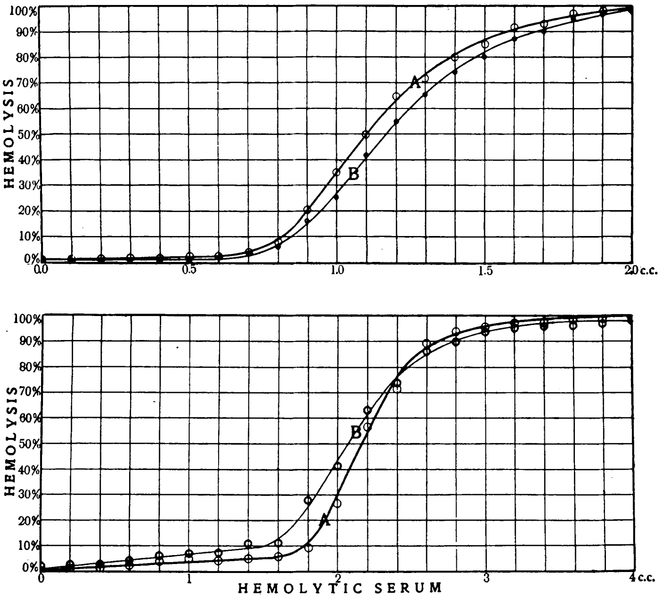

FIG. 6.—HEMOLYTIC SUSCEPTIBILITY OF CORPUSCLES AFTER EXPOSURE TO THIRD COMPONENT.

Curves, as in Fig. 5, showing changes in hemolytic susceptibility, after exposure to third component, that are not accounted for by the experimental error. In the upper experiment, the corpuscles were exposed to an auxilytic third component; in the lower experiment, to an antilytic third component. In each case the slight observed changes are the opposite of those that would have been expected from an absorption of the third component in question. The meaning of these changes is not clear, but they evidently are not changes due to third component absorption.

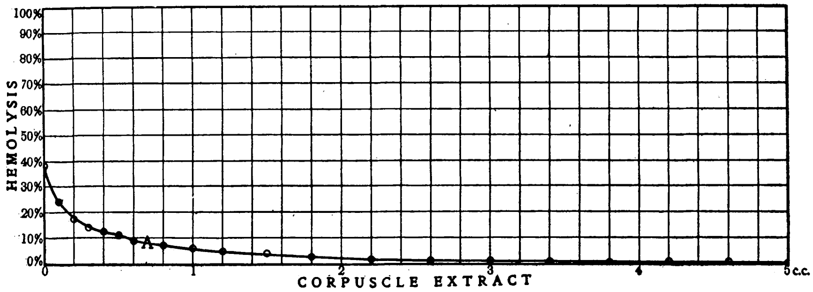

FIG. 7.—ANTILYTIC ACTION OF CORPUSCLE EXTRACT.

Curve showing changes in hemolytic power produced by adding increasing amounts of corpuscle extract (exposed salt solution) to a constant amount of hemolytic serum. The constant hemolytic serum used in this experiment was capable, in itself, of producing 38 per cent hemolysis.

they are completely removed by subsequent washing in salt solution. Absorption is experimentally undemonstrable.

In order to test the second hypothesis, that hemolytically active substances are given off from the corpuscles into the third component, washed corpuscles were exposed to physiological saline (0.85 per cent NaCl), under conditions identical with those of the amboceptor absorption experiments above, the salt solution simply taking the place of the heated serum. The salt solution was then freed from corpuscles by centrifugation, and its effect on hemolysis tested.

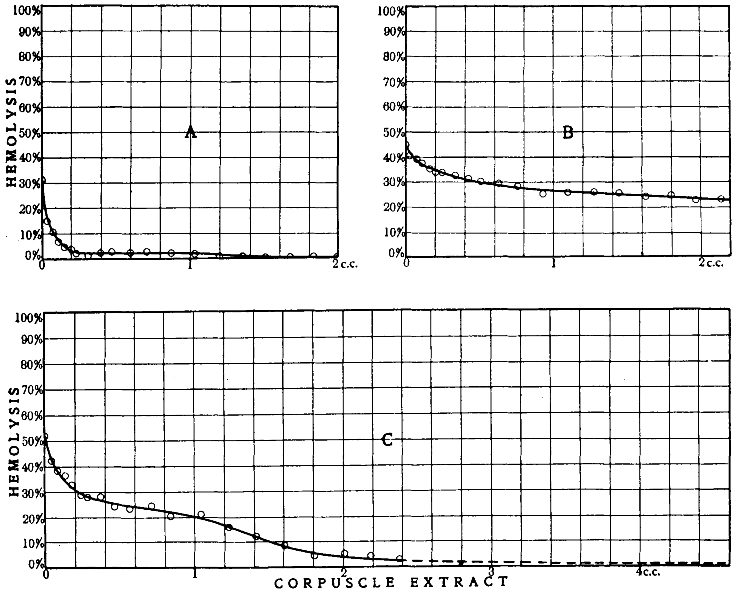

FIG. 8.—ANTILYTIC ACTION OF CORPUSCLE EXTRACT.

Curves, as in Fig. 7, showing changes in hemolytic power produced by adding increasing amounts of three different corpuscle extracts to different constant amounts of hemolytic serum.

It was found that this exposed salt solution (corpuscle extract) was in all cases powerfully antilytic. Such antilytic effects are shown graphically in Figs. 7, 8, and 9.

Corpuscles, therefore, give off a hemolytically active substance into salt solution. Is this substance also given off into third component? If so, and if the giving off of this substance is the only change brought about by corpuscles in exposed third component, it

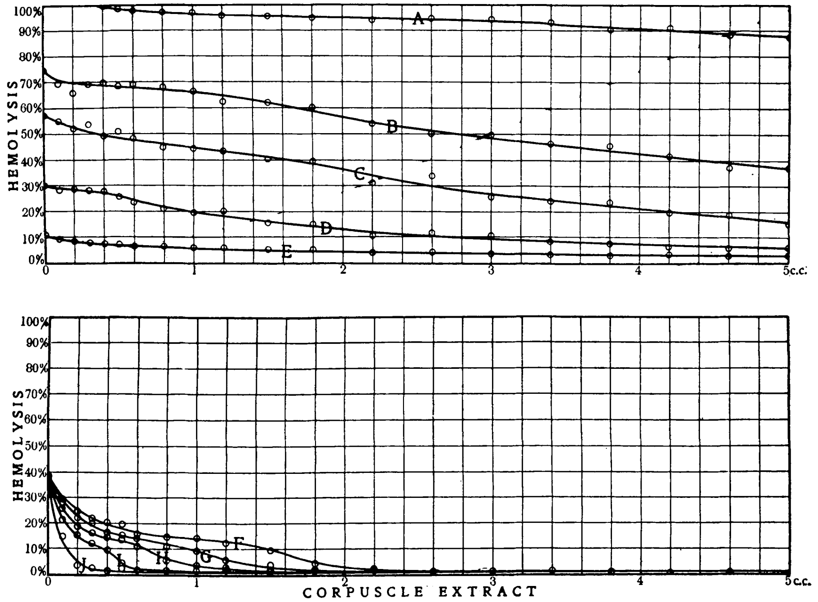

FIG. 9.—ANTILYTIC ACTION OF CORPUSCLE EXTRACT.

Curves, as in Figs. 7 and 8, showing changes in hemolytic power produced by adding increasing amounts of corpuscle extract, to constant amounts of hemolytic serum. *A*, *B*, *C*, *D*, and *E*=curves obtained with the same extract added to different constant amounts of the same hemolytic serum. *F*, *G*, *H*, *I*, and *J*=curves obtained with extracts from different numbers of the same corpuscles, added to a constant amount of the same hemolytic serum.

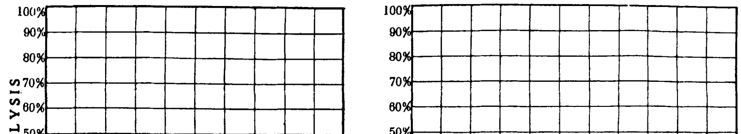

FIG. 10.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Two sets of curves showing different effects on third component, of the addition of corpuscle extract. *A*=curve obtained with unexposed third component. *B*=curves with exposed third component. *M*=curves with corpuscle extract. This extract was in both cases obtained under the same experimental conditions, as those used in obtaining the exposed third component, salt solution simply taking place of the heated normal serum. *X*=curves obtained with a mixture of equal volumes of corpuscle extract (*M*) and unexposed third components. *Y*=curve obtained by adding a half-volume of corpuscle extract (*M*) to the unexposed third component.

In the first set of curves the addition of an equal volume of corpuscle extract produced a more marked change in the third component than the change produced in the same serum by exposure to corpuscles. In the second set of curves, the addition of an equal volume of extract produced a less marked change than such exposure.

should be possible to produce artificially the observed third component changes, by simply adding corpuscle extract to unexposed third component.

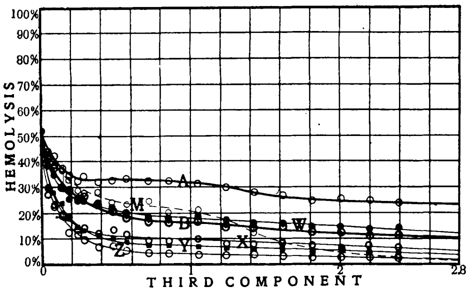

FIG. 11.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Curves, as in Fig. 10, showing the effect on the third component of the addition of corpuscle extract. *A* = curve with unexposed third component. *B* = curve with exposed third component. *M* = curve with corpuscle extract. *W* = curve obtained by adding a half-volume of corpuscle extract (*M*) to unexposed third component. *X* = curve with the addition to an equal volume of extract. *Y* = curve with one and a half volumes of extract. *Z* = curve with twice the volume of extract. In this experiment the nearest approach to the exposed third-component curve was produced by the addition of a half-volume of extract (see Fig. 14).

To test this possibility, various mixtures of unexposed third component and corpuscle extract were made, and the action of these mixtures compared with that of third component exposed to corpuscles. Four sets of data thus obtained are shown in Figs. 10, 11, and 12.

The essential curves from these figures have been grouped together in Fig. 13. From this figure it is seen that, in two of the experiments (I and III), the addition of an equal volume of corpuscle extract to unexposed third component, conferred on the component greater antilytic powers than that acquired by exposure to corpuscles. In a third experiment (II), the addition of an equal volume conferred less auxilytic powers; while in a fourth experiment (IV), it gave greater antilytic power when the component was tested in certain amounts, but less antilytic power when tested in other quantities.

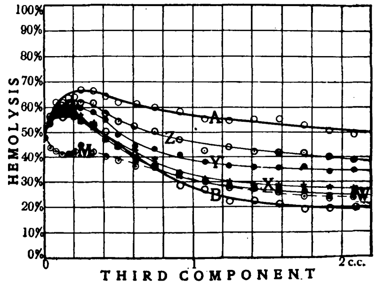

FIG. 12.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Curves, as in Figs. 10 and 11, showing the effect on the third component of the addition of corpuscle extract. *A*, *B*, and *M* = curves with unexposed third component, exposed third component, and corpuscle extract, as before. *Z* = curve obtained by the addition of a quarter-volume of corpuscle extract. *Y* = curve with a half-volume. *X* = curve with a three-quarter volume. *W* = curve with an equal volume. In this experiment, the nearest approach to the exposed serum is made by the addition of an equal volume of corpuscle extract.

The nearest artificial approximation to an exposed third component was obtained, in one of the experiments (III), by adding a half-volume of corpuscle extract. The nearly coincident curves from this experiment are shown in Fig. 14.

The addition of corpuscle extract to third component, therefore, produces changes in the third component, approximating those produced by exposure to corpuscles, but not identical with them. If we assume that the changes produced in exposed third component are mainly due to the giving off of corpuscle products into that component, it will be necessary to assume that the amount of these products so given off is influenced by the nature of the third component used; that with one third component there is given off an amount equal to that given off by the same corpuscles into physiological saline; that with another third component a much smaller amount is given off than in the corresponding experiment with physiological salt solution; while with a third experiment a much larger amount is given off.

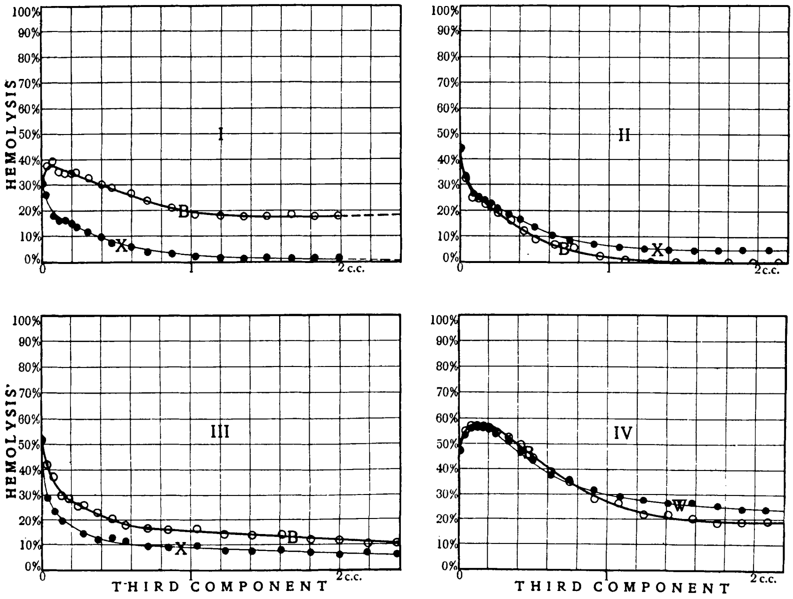

FIG. 13.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Curves obtained with the addition of equal volumes of corpuscle extract, brought together from FIGS. 10, 11, and 12, above.

Moreover, this hypothesis would still leave unaccounted-for certain minor changes in the third component, indicated by the crossing of the approximating curves. What this change is, and whether or not it can be accounted for by a hypothetical digestion of third component by corpuscle enzymes, is still a matter of pure conjecture.

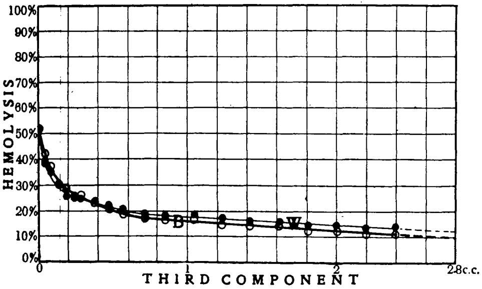

FIG. 14.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

The nearly coincident curves of Fig. 11.

The qualitative changes produced in the third sreum component by exposure to corpuscles are therefore such that they can neither be predicted nor reproduced artificially with sufficient accuracy for analytical purposes. This being the case, it does not seem possible at present to devise an indirect method of analysis by means of which the absorption of hemolytic amboceptor can be determined.

In the light of this conclusion, it would seem desirable to examine with care fundamental analytical data in other fields of serum pathology, to determine whether or not they rest on a sufficiently reliable foundation for the theoretical deductions now made from them.

SUMMARY.

1. Heated normal goat serum (pure third serum component) is altered in its chemical nature by exposure to washed sheep corpuscles. A third component that is originally auxilytic has its auxilytic powers decreased by such exposure, while a third component originally antilytic has its antilytic powers increased.

2. Absorption of the third serum component by the exposed sheep corpuscles is not demonstrable.

3. Washed sheep corpuscles give off into exposed physiological saline a powerful antilytic substance.

4. The addition of this antilytic corpuscle extract to unexposed third component produces approximately the same changes in the third component as those produced by exposure to corpuscles. The amount of extract necessary to produce the desired change, however,

varies with different sera. With one third component it may be the same amount as that given off by duplicate corpuscles, under identical experimental conditions, into physiological saline. With another third component, it may be a much smaller amount, and with a third serum, a much larger amount.

5. If we assume that the changes taking place in exposed third component are mainly due to the addition of corpuscle products, it will be necessary to assume certain factors in the third component that influence the amount of these products so acquired. In the presence of one third component, the giving off of these products by the corpuscles must be assumed to be stimulated; with another third component, retarded; while with a third it is not influenced.

6. Moreover, distinct differences between experimental curves indicate, that, in addition to the giving off of corpuscle products into third component, there must be assumed certain minor secondary changes in the third component, the nature of which is still a matter of conjecture.

7. The changes in exposed third component are so complex, that an indirect method of determining the amboceptor absorption by sheep corpuscles seems at present impossible.